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NKTR-214 (CD122-biased agonist) and NKTR-262 (TLR7/8 agonist) combination treatment pairs local innate immune activation with systemic CD8+ T cell expansion to enhance anti-tumor immunity

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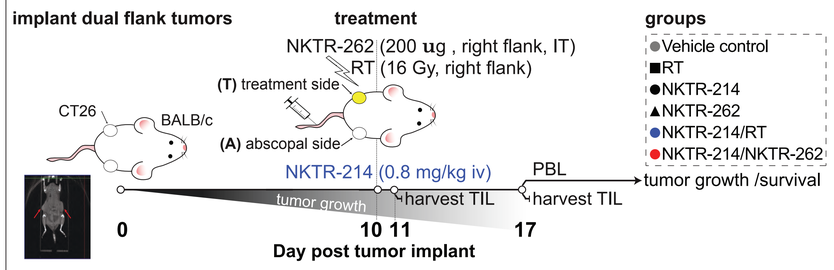
Introduction

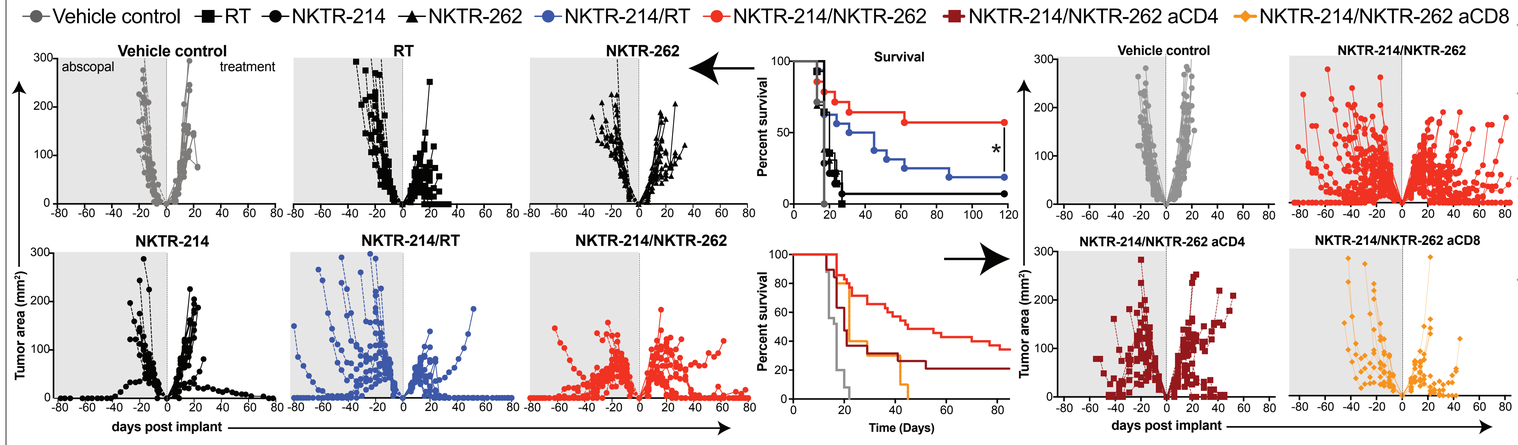
- The abscopal effect refers to the ability of localized radiation to trigger systemic anti-tumor effects. However, with radiation therapy (RT) alone, the abscopal effect is exceedingly rare.
- Combining local RT with immunotherapy such as NKTR-214, a CD122-biased cytokine agonist conjugated with releasable polyethylene-glycol (PEG) chains, increases abscopal response rates, but still leaves room for improvement.
- The biologic mechanism driving the abscopal response is yet to be fully understood, but immunosuppression is throught to contribute to low abscopal response rates.
- Toll-like receptor (TLR) signaling can induce APC differentiation and reduce tumor microenvironment immune suppression.
- We hypothesized that combination treatment with a novel TLR7/8 targeting agent, NKTR-262, with NKTR-214 will activate innate and adaptive anti-tumor immune responses that will increase abscopal tumor responses.
- Finally, comparison of NKTR-214 combined with either RT or NKTR-262, will reveal mechanisms driving abscopal responses.

Methods



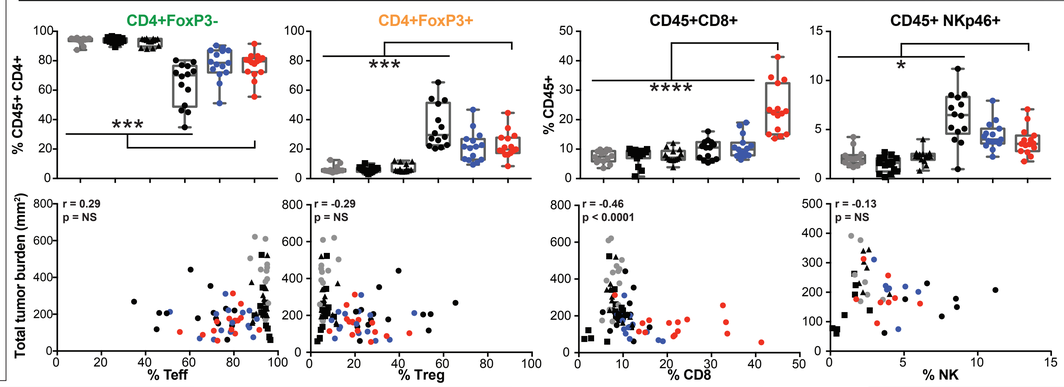
- Using flow cytometry we evaluated CD4+, CD8+, and NK cell activation status in the blood and tumor 7 days post-treatment and we evaluted innate immune subsets (macrophages, monocytes) including M1/M2 polarization 1 day post-treatment. To determine the contribution of each cell type, mice were treated with depleting antibodies 1x/week.

1 NKTR-214/NKTR-262 treatment improves abscopal responses through a CD8 T cell dependent mechanism



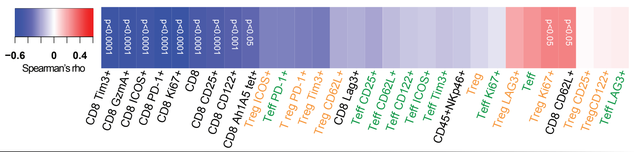
- Mice bearing dual flank CT-26 tumors were treated as described in the methods.
- Tumor area (mm²) was determined every 2-3 days on the treatment and abscopal sides (grey boxes).
- NKTR-214/NKTR-262 combination treatment significantly reduced tumor growth and increased survival (top center).
- This response depends primarily on CD8 cells as depletion of CD8s (yellow diamond) but not CD4 (maroon square), abrogated the effect. N>10 from two independent experiments.

2 Peripherial blood CD8 T cell responses correlate with overall tumor burden



- Vehicle control ■RT ●NKTR-214 ▲NKTR-262 ●NKTR-214/RT ●NKTR-214/NKTR-262
- 7 days post treatment, peripherial blood was collected, and T cell phenotypes were determined by flow cytometry (top row). Statistical significance was determined using a 1-way ANOVA comparing all groups against NKTR-214/NKTR-262, with a p-value cut-off of 0.05.
- Cell percentages were compared to total tumor burden using Spearman correlation to identify which cell types may impact tumor size (bottom row and heat map).
- Nearly all CD8 cell phenotypes in the periphery correlate with total tumor burden 7 days post treatment (heat map below). N>12 from two independent experiments.

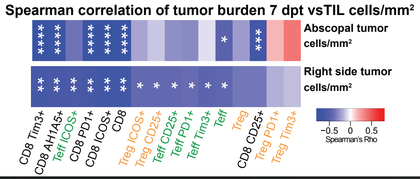
Spearman correlation between tumor burden 7 d post treatment and identified cell type

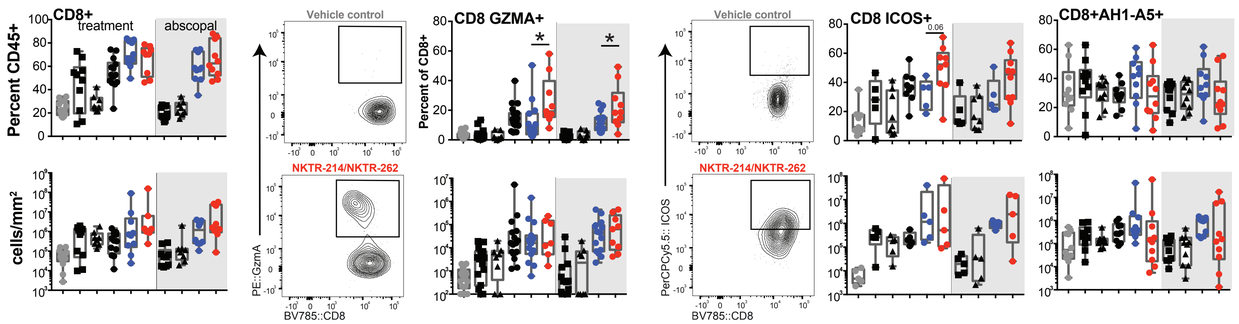


3 Intratumoral CD8 T cell responses correlate with tumor burden

- Data is presented as percent of population (top row) and cell density (bottom row). Statistical significance was determined using Students T Test between the two combination groups.
- Cell densities were compared to tumor burden for the treated and abscopal tumor (heat map below).
- CD8 T cell responses correlate strongly with abscopal tumor size

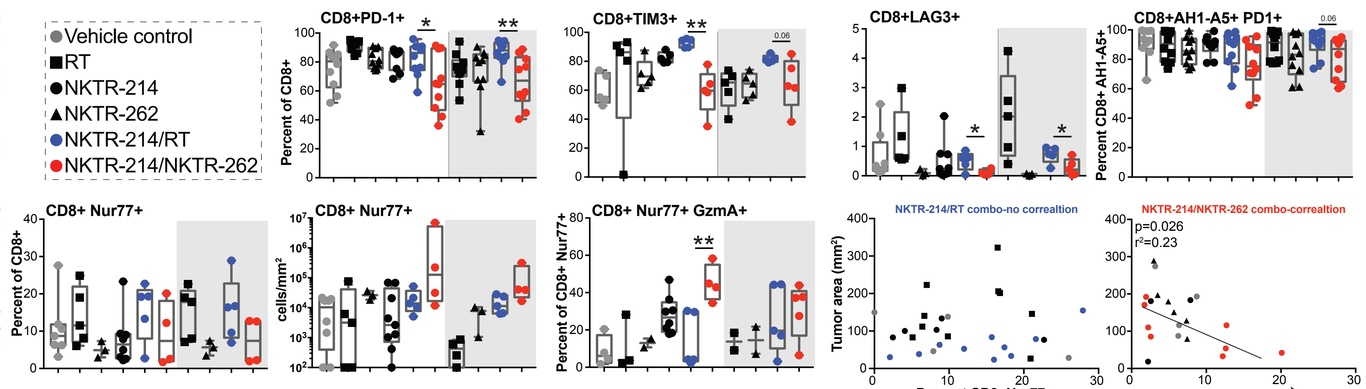
Spearman correlation of tumor burden 7 dpt vsTIL cells/mm²



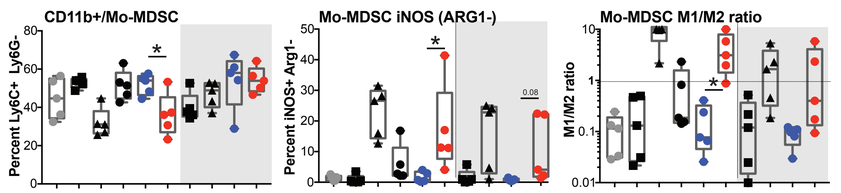


4 NKTR-214/NKTR-262 induces less exhausted and more active CD8 T cell responses

- NKTR-214/NKTR-262 combination treatment resulted in CD8 T cells with reduced expression of exhaustion markers PD-1, Tim3, and Lag3. Further, tumor reactive CD8 T cells (AH1-A5+) also had reduced expression of PD-1. N>8 from two independent experiments.
- Statistical significance was determined using Students T Test between the two combination groups.
- Nur77:GFP+ mice bearing dual flank CT-26 tumors treated with NKTR-214/NKTR-262 had a greater tumor CD8+ Nur77+ cell density, which also expressed increased GzmA levels.
- NKTR-214/NKTR-262 induces more functional cells, which is represented by a relationship between those cells and tumor size in the NKTR214/NKTR-262 treated mice. N=4 from one expt.



5 NKTR-214/NKTR-262 TME favors M1 monocytes



- Data is presented as percent of population or a ratio of %M1/%M2 based on iNOS and Arg1 expression. N>4 from one experiment.
- NKTR-214/NKTR-262 combination treatment resulted in monocytic cells (CD11b+Ly6C+Ly6G-) with increased iNOS expression, and by extention an increased M1/M2 ratio.
- The data suggests that NKTR-262 may alter the TME to be less suppressive, thereby supporting a more effective adaptive response.

Conclusions

- NKTR-214/NKTR-262 significantly increases the abscopal responses in comparison to NKTR-214/RT combination therapy in a CD8 T cell dependent manner.
- Across all treatment groups, peripherial CD8 T cells correlate with tumor burden 7 days post treatment.
- NKTR-214/NKTR-262 combination treatment results in an increased density of CD8 T cells in both the treated and abscopal tumor. These CD8 T cells have increased GzmA expression and reduced conversion to an exhausted phenotype (PD-1, Tim3, Lag3).
- These active CD8 T cells are supported by a less suppressive tumor microenvironment, demonstrated by the increased M1/M2 monocytic ratio in NKTR-214/NKTR-262 treated animals.

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